Effect of Nisin-EDTA, Chlorine and Hydrogen Peroxide Treatments in Reducing Transfer of Naturally Occurring Microflora of Whole Melon to Fresh-cut Pieces

Dike O. Ukuku, Gerald Sapers and William F. Fett

U.S. Department of Agriculture†, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038

Key words: whole melon, fresh-cut, washing treatment, pathogen attachment, survival, transfer

ABSTRACT

Efficacy of nisin-EDTA treatment as a sanitizer for reducing populations and transfer of native microflora of whole melons to fresh-cut melons was compared to that of chlorine(200 ppm) and hydrogen peroxide(2.5%) treatments. Whole cantaloupe and honeydew melons were washed with water, nisin (10 μg/ml)-EDTA (0.02 M), 200 ppm chlorine or hydrogen peroxide (2.5%) for 5 min at ~ 22 °C before fresh-cut preparation. Preliminary studies indicated that water washes, EDTA (0.002 to 0.2 M) or nisin (10 μg/ml) were not effective in reducing the populations of native microflora of whole melon when used individually. The predominant class of organisms on cantaloupe and honeydew melon were aerobic mesophilic bacteria followed by lactic acid bacteria, total gram-negative bacteria, yeast and mold and Pseudomonas *spp. Nisin-EDTA, chlorine and hydrogen peroxide treatments were significantly ($p<0.05$) more effective in reducing native microflora than water washes. Nisin-EDTA treatments were significantly ($p<0.05$) more effective than chlorine or hydrogen peroxide in reducing populations of yeast and mold and* Pseudomonas *spp. on whole melon surfaces, but were not as effective as chlorine or hydrogen peroxide treatments for reducing aerobic mesophilic bacteria, lactic acid bacteria and total gram-negative bacteria. Although the total microbial population on the surfaces of cantaloupe and honeydew melons was significantly reduced by the nisin-EDTA treatment, the results suggest that treatment with nisin-EDTA before fresh-cut processing is not as effective as hydrogen peroxide and chlorine in reducing transfer of native microflora of whole melon to fresh-cut pieces.*

1.0 INTRODUCTION

Cantaloupes and other melons have been associated with numerous outbreaks of foodborne illness in recent years (NACMCF 1999; DeWaal *et al.* 2000). The causative organisms in cantaloupe-related outbreaks to date were *Salmonella* serovars including

---

†Mention of trade names or commercial products in this [article] [publication] is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Chester and Poona (Tamplin 1997; NACMCF 1999). Other human pathogens including *E. coli* O157:H7 and *Shigella* are capable of growth on melon flesh (Golden *et al.* 1993; Del Rosario and Beuchat 1995). The recent FDA survey of imported fresh produce reported an incidence of 5.3% positives for *Salmonella* and 2.0 % for *Shigella* in 151 samples of cantaloupes, all contaminated samples originating in Mexico, Costa Rica and Guatemala (FDA 2001a). The FDA reported 2.6 % positives for *Salmonella* and 0.9 % for *Shigella* in 115 samples of cantaloupes in a survey of domestic fresh produce (FDA 2001b).

Contamination most likely originates directly or indirectly from fecal matter either pre- or postharvest and may involve use of contaminated irrigation water or uncomposted manure. Contributing factors can include poor hygiene and unsanitary procedures of field and processing workers, inadequate cleaning of processing equipment, the use of decayed or damaged melons, and failure to wash melon properly before fresh-cut processing (Brackett, 1992; Brackett, 1994).

Physical and chemical treatments are used in food processing to eliminate or at least reduce the presence of pathogenic and spoilage microorganisms (Wei *et al.* 1985; Ray 1992). Chlorination of wash water up to 200 ppm is routinely applied to reduce microbial contamination in produce processing lines (Wei *et al.* 1985). However, the use of chlorine is of concern due to the potential formation of harmful by-products (Richardson *et al.* 1998) and can only achieve approximately 2 to 3 log reductions of native microflora (Ayhan et al. 1998;Ukuku *et al.* 2001). Thus, there is much interest in developing safer and more effective sanitizers.

We have previously investigated use of a hydrogen peroxide wash for reducing attached bacteria on whole cantaloupe surfaces (Ukuku *et al.* 2001) and minimally processed fruits and vegetables (Sapers and Simmons 1989; Ukuku and Sapers, 2001). Efficacy of hydrogen peroxide in preservation of fresh fruits and vegetables (Honnay 1998), and preservation of fresh-cut melons (Sapers *et al.* 2001) has been reported. There are several reports that nisin used in combination with a chelating agent exhibits a bactericidal effect towards both gram-positive and gram-negative bacteria (Blackburn *et al.* 1989; Cutter and Siragusa 1995; Stevens *et al.* 1991, 1992a,b). In this study we examined and compared the efficacy of nisin-EDTA treatments to that of hydrogen peroxide and chlorine in reducing surface populations and transfer of indigenous microflora of whole cantaloupes and honeydew melons to the flesh during fresh-cut preparation.

2.0 MATERIALS AND METHODS

2.1 *Cantaloupe and honeydew Melons*

Cantaloupe (1720 ± 34 g) and honeydew (1837 ± 34 g) melons were purchased from a local wholesale distribution center. Melons were stored at 4°C. Before use, melons were unpacked and placed on the laboratory bench for ~24 h to allow them to come to room temperature (~22°C).

2.2 *Preparation of sanitizing solutions*

Clorox, a commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox Company, Oakland CA), was diluted with sterile water to provide a concentration of 200 ppm of available chlorine in the wash solution. The pH was adjusted to 6.4 ± 0.1 by adding citric acid. Free chlorine in the solution was then determined with a chlorine test kit (Hach Co., Ames, IA) that has been approved by the U.S. Environmental Protection Agency. A 2.5 % hydrogen peroxide solution was prepared from a 30% stock solution (Reagent grade, Fisher Scientific, Suwanee, GA) by diluting with deionized distilled water (ddH_2O). A stock solution of nisin (10^6 I. U., Sigma, St. Louis, MO) was prepared at a concentration of 2500

μg/ml in 0.02 N hydrochloric acid (HCl, pH 2). The stock solution was filter sterilized (0.22 μm, Millipore, Bedford, MA), and stored at –80° C until used. A stock solution of 2 M disodium EDTA (Fisher Scientific Co., Pittsburgh, PA) was prepared in ddH_2O, autoclaved at 121°C for 15 min, and then stored at room temperature until used. For use, the stock solutions of disodium EDTA was diluted with sterile tap water to give a final concentration of 0.02M and the stock solution of nisin was dissolved in 0.02 M EDTA to give a final concentration of 10 μg/ml.

2.3 *Sanitizing treatments*

Surfaces of whole melons were treated with water, 200 ppm chlorine, 2.5% H_2O_2, nisin (10 μg/ml), EDTA (0.02 M) or nisin (10 μg/ml)-EDTA (0.02 M) combination to reduce bacterial populations on the surface of cantaloupe. All washing treatments were performed by submerging the melons under the surface of 3 L sterile wash solution for 5 min. Washed melons were allowed to dry for 1 h in a biosafety cabinet before fresh-cut preparation and microbiological analysis.

2.4 *Preparation of fresh-cut pieces*

To prepare fresh-cut pieces, treated and untreated whole cantaloupes or honeydew melons were cut into four sections using a sterile knife and the rinds carefully removed. The interior flesh was cut into ~3 cm cubes, and the melon pieces (100 g) were placed inside a 64 oz round plastic tub (Mac# 7) (Rock Tenn. Co., Franklin Park, IL). The plastic tubs containing the pieces were stored at 5°C for up to 15 days.

2.5 *Microbiological analyses*

A sterilized stainless steel cork-borer was used to cut through the cantaloupe or honeydew rind at random locations to produce rind plugs of 22 mm in diameter with a rind surface area (πr^2) of 3.80 cm^2. Flesh adhering to the rind plugs was trimmed off using a sterilized stainless steel knife. Forty rind plugs per whole melon weighing approximately 20 g were blended (Waring commercial blender, Dynamic Corp, New Hartford, CT, speed set at level 5) for 1 min with 80 ml of sterile 0.1% peptone water. Decimal dilutions of the sample were made with 0.1% peptone water, and aliquots (0.1 ml) were plated in duplicate on a range of media. Plate Count Agar (PCA, Difco Becton Dickinson, Sparks, MD) with incubation at 30°C for 3 days was used for enumeration of mesophilic aerobes. PCA + crystal-violet (2 mg/ml) with incubation at 30°C for 3 days was used for enumerating gram-negative bacteria (Cousin *et al.* 2001). *Pseudomonas* spp. were enumerated by plating 0.1 ml on *Pseudomonas* Isolation Agar (Difco) with incubation at 27°C for 3 days. Lactic acid bacteria were enumerated with de Man, Rogosa and Sharpe agar (MRS, Oxoid, Ogdensburg, New York) with 0.08% sorbic acid as a supplement with incubation at 30C for 3 days (Reuter 1985). Yeast and mold were enumerated according to Norris and Ribbons (1971) using Czapek Malt Agar (CMA, Sigma, St. Louis, MD) with incubation at 25°C for 5 days.

For fresh-cut pieces before or after storage, 200 ml of 0.1% peptone water was added to Stomacher® bags containing fresh-cut pieces (100 g/bag) and the bag contents pummeled for 30 s in a Stomacher® model 400 (Dynatech Laboratories, Alexandria, VA) at medium speed. Homogenates of zero time samples, and samples stored for 3 days at 5°C were plated (0.1 ml) on the different agar media as stated above. For fresh-cut pieces stored for more than 3 days, decimal serial dilutions prepared in 0.1% peptone water were plated on the agar media.

3.0 *Statistical analyses.*

All experiments were done in triplicate with duplicate samples analyzed at each sampling time. Data were subjected to the Statistical Analysis System (SAS; SAS Institute, Cary, NC) for analysis of variance (ANOVA) and the Bonferroni LSD method to determine if there were significant differences ($p<0.05$) between mean values of number of cells recovered after each treatment.

4.0 RESULTS

4.1 *Effect of sanitizing treatments on microflora of whole melon.*

Initial populations of native microflora on the rind of unwashed whole cantaloupes and honeydew melons are shown in Table 1. The surface of cantaloupe melon supported higher populations of all classes of microbes than the surface of honeydew melon. The predominant class of organisms on cantaloupe and honeydew melon were aerobic mesophilic bacteria followed by lactic acid bacteria and total gram-negative bacteria. Populations of yeast and mold and *Pseudomonas* spp. were the lowest.

TABLE 1.
EFFECT OF NISIN-EDTA, CHLORINE AND HYDROGEN PEROXIDE TREATMENTS ON THE NATIVE MICROFLORA OF WHOLE MELONS

Treatment	Survivors (log_{10} CFU/cm^2)				
	APC[b]	Gram-negative bacteria	LAB[b]	Yeast & Mold	*Pseudomonas* spp.
Cantaloupe					
Control	6.82 ± 0.23^a	3.10 ± 0.10^a	4.40 ± 0.15^a	2.38 ± 0.15^a	2.04 ± 0.13^a
Water	6.60 ± 0.14^a	3.00 ± 0.14^a	4.32 ± 0.13^a	2.03 ± 0.12^a	2.00 ± 0.10^a
Cl_2 (200 ppm)	3.38 ± 0.15^c	1.24 ± 0.11^c	1.34 ± 0.12^b	0.92 ± 0.10^b	1.76 ± 0.11^b
Nisin-EDTA[c]	4.55 ± 0.14^b	2.39 ± 0.12^b	4.17 ± 0.14^a	0.42 ± 0.12^c	0.95 ± 0.06^c
H_2O_2 (2.5%)	3.02 ± 0.10^c	0.98 ± 0.11^c	1.18 ± 0.12^b	0.98 ± 0.11^b	1.42 ± 0.10^b
Honeydew					
Control	3.51 ± 0.16^a	2.30 ± 0.13^a	2.28 ± 0.14^a	1.04 ± 0.09^a	1.30 ± 0.13^a
Water	3.40 ± 0.14^a	2.24 ± 0.10^a	2.32 ± 0.11^a	0.89 ± 0.04^b	1.26 ± 0.10^a
Cl_2 (200 ppm)	1.30 ± 0.15^c	0.69 ± 0.13^c	0.49 ± 0.12^c	0.57 ± 0.10^c	0.76 ± 0.11^b
Nisin-EDTA[c]	2.07 ± 0.10^b	1.45 ± 0.11^b	2.07 ± 0.10^a	0.22 ± 0.12^d	0.35 ± 0.08^c
H_2O_2 (2.5%)	1.00 ± 0.10^c	0.43 ± 0.11^c	0.36 ± 0.11^c	0.30 ± 0.10^c	0.48 ± 0.12^b

[a]Values represent means for data from three experiments with duplicate determinations per experiment. Means in the same column for each type of melon not followed by the same letter are significantly ($p<0.05$) different.
[b]APC = Aerobic mesophilic bacteria, LAB = Lactic acid bacteria.
[c]Nisin-EDTA = 10 μg/ml nisin in 0.02 M EDTA

Washing with water, EDTA or nisin alone did not cause significant reductions of all groups of microorganisms tested for on the outer surfaces of cantaloupe and honeydew melon.

Combinations of EDTA and nisin were tested as a washing treatment and compared to chlorine (200 ppm) and hydrogen peroxide (2.5%) washes. The combination treatments with nisin (10 μg/ml)-EDTA (0.02 M) were significantly ($p<0.05$) more effective than washing with water alone for reducing aerobic mesophilic bacteria, gram-negative bacteria, *Pseudomonas* spp. and yeast and molds, but not lactic acid bacteria. The chlorine and hydrogen peroxide treatments were significantly ($p<0.05$) more effective than the nisin-EDTA combination treatment against all groups of microbes except *Pseudomonas* spp. and yeast and mold (Table 1). For these two categories of microbes, nisin-EDTA treatment was significantly ($p<0.05$) more effective. Washing with nisin-EDTA reduced the total aerobic mesophilic bacteria on the cantaloupe rinds by 2.3 $\log_{10}$ CFU/cm^2 as compared to 3.4 and 3.8 $\log_{10}$ reductions for the chlorine and hydrogen peroxide treatments, respectively. The nisin-EDTA treatment reduced gram-negative and lactic acid bacteria on the cantaloupe rinds by 0.7 $\log_{10}$ CFU/cm^2 and 0.2 $\log_{10}$ CFU/cm^2 respectively, while yeast and mold were reduced by 2.0 $\log_{10}$ CFU/cm^2. Overall, the data indicated that hydrogen peroxide and chlorine treatments were more effective in reducing the native microflora on the cantaloupe rind than the nisin-EDTA treatment. Similar results were observed for honeydew melon (Table 1).

4.2. *Transfer of native microflora from whole melon to fresh-cut pieces.*

Fresh-cut pieces prepared from whole untreated melons were also found to harbor similar classes of microbes, but at lower populations (Figure 1). Immediately after cutting, the population for total mesophilic aerobic bacteria determined on fresh-cut pieces of cantaloupe and honeydew melon were 3.33 and 1.07 log CFU/g, respectively. Populations of lactic acid bacteria determined on fresh-cut cantaloupe and honeydew melon were 2.08 log CFU/g. Populations of gram-negative bacteria, yeast and mold and *Pseudomonas* spp. on fresh-cut cantaloupe and honeydew pieces were very low.

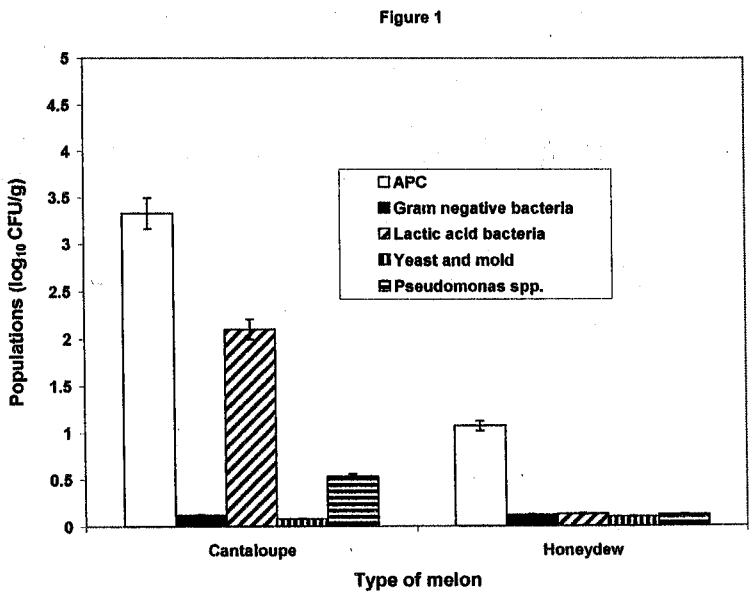

FIGURE 1. *Populations of native microflora on fresh-cut melon pieces immediately after preparation. Values are mean ± standard deviation of three experiments with duplicate determinations.*

4.3. *Effect of sanitizer treatments and storage (5°C) on the population of transferred native microflora in fresh-cut pieces*

Populations of native microflora of cantaloupe fresh-cut pieces prepared from treated and untreated whole melons stored at 5°C for up to 15 days is shown in Figure 2. Overall, fresh-cut pieces prepared from whole cantaloupe melons washed with hydrogen peroxide had the lowest microbial population followed by fresh-cut pieces from chlorine and nisin-EDTA treated whole melons. Populations for *Pseudomonas* spp. and yeast from fresh-cut pieces prepared from hydrogen peroxide treated whole melon were less than 2.0 log CFU/g at the end of 15 days storage at 5°C. Populations of all groups of microorganisms including *Pseudomonas* spp. increased in all samples as storage time increased, regardless of the treatment, but populations of the native microflora on the fresh-cut pieces prepared from sanitized whole melons remained lower throughout the storage period.

Figure 2-Cantaloupe

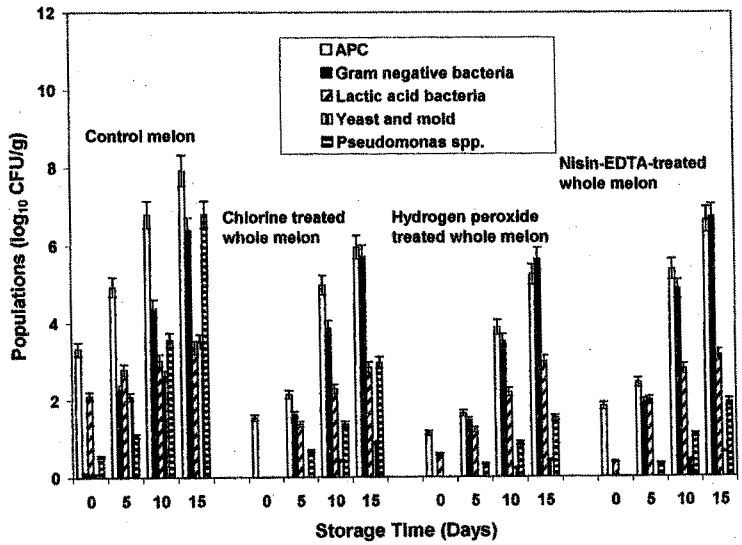

FIGURE 2. *Effect of sanitizing treatments and refrigerated (5°C) storage on survival of native microflora in fresh-cut cantaloupe pieces. Values are mean ± standard deviation of three experiments with duplicate determinations. Where no data is shown, populations were below the level of detection.*

Native microflora of honeydew fresh-cut pieces stored at 5°C for up to 15 days is shown in Figure 3. Overall, the results were similar to those found for cantaloupe melons with the hydrogen peroxide wash being the most effective in reducing microbial populations.

Figure 3-Honeydew

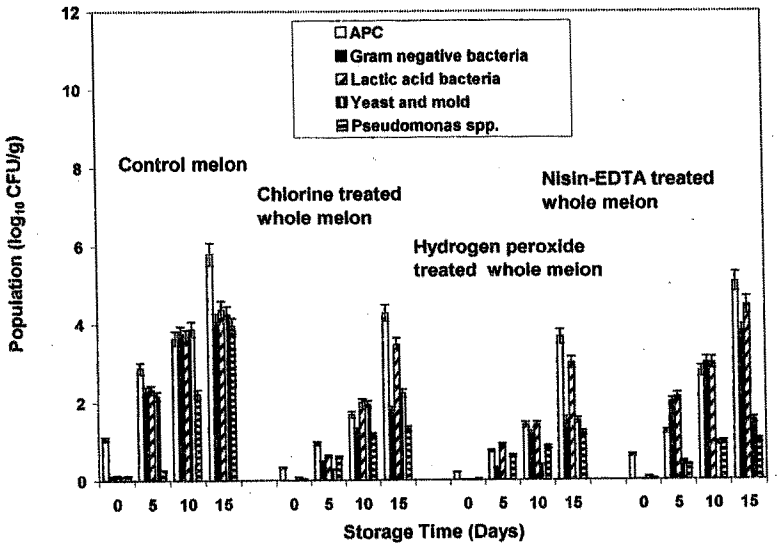

FIGURE 3. *Effect of sanitizing treatments and refrigerated (5°C) storage on survival of native microflora in fresh-cut honeydew pieces. Values are mean ± standard deviation of three experiments with duplicate determinations. Where no data is shown, populations were below the level of detection.*

5.0 DISCUSSION

The fact that the same categories of microorganisms were detected on whole melon surface and on fresh-cut pieces during storage indicates that the microbes were transferred from the rind to the flesh during fresh-cut preparation. The differences in the populations of the native microflora on the honeydew and cantaloupe rind are most likely due to the rough surface of the cantaloupe rind compared to the relatively smooth surface of honeydew melon (Ukuku and Fett, 2002). The extensive raised netting on the surface of cantaloupe melon no doubt provides more microbial attachments sites and helps to protect attached microbes from being washed from the surface, and possibly from environmental stresses such as UV radiation and desiccation.

Washing with water, EDTA or nisin alone did not cause significant reductions of all groups of microorganisms tested for on the outer surfaces of cantaloupe and honeydew melon. However, treatments with a combination of nisin and EDTA did result in significant reductions, but overall, were not as effective as the H_2O_2 and chlorine washes. A synergistic antibacterial activity of nisin and EDTA against soft rotting *Erwinia carotovora*, *E. chrysanthemi*, *Pseudomonas virdiflava* and *P. fluorescens* when cultured in trypticase soy broth has been reported (Wells *et al.* 1998). However, nisin-EDTA treatments were not effective in reducing bacterial soft rot on cut carrot slices. Nisin-chelator treatment of red meat delayed growth of *Staphylococcus aureus* and *Listeria monocytogenes* for a day at room temperature and for up to 2 weeks at 5 °C (Chung *et al.* 1989). Sanitizer treatments with H_2O_2 and chlorine were similar in efficacy. The results of this study suggest that the use of alternative sanitizers such as nisin-EDTA and H_2O_2 might be viable alternative to chlorine for decontaminating cantaloupe or other melon surfaces before fresh-cut preparation.

REFERENCES

1. Adams, M. R., Hartley, A. D. and Cox, L. J. 1989. Food Microbiol. *6*, 69-77.
2. Ayhan, Z., Chism, G.W. and Richter, E.R. 1998. J. Food Quality *21*, 29-40.
3. Blackburn, P., Polack, J., Gusik, S. and Rubino, S.D. 1989. Applied Microbiology, Inc., New York.
4. Benkerroum, N. and Sandine, W.E. 1988. J. Dairy Sci. *71*, 3237-3244
5. Beuchat, L. R. 1995. J. Food Prot. *59*, 204-216.
6. Brackett, R. E. 1992. J. Food Prot. *55*, 808-814.
7. Brackett, R. E. 1994. p. 269-312. *In* R.C. Wiley, (ed.), Minimally Processed Refrigerated Fruits and Vegetables. Chapman Hall, New York.
8. Chung, K-T., Dickson, J.S., and Crouse, J.D. 1989. Appl. Environ. Microbiol. *55*, 1329-1333.
9. Cousin, M.A., Jay, J.M. and Vasavada, P.C. 2001. *In* F.P Downes and Ito, K. (eds.). Amer Pub. Health Assoc. Washington, D.C.
10. Cutter, C.N., and Siragusa, G.R. 1995. J. Food Prot. *58,* 977-983.
11. Del Rosario, B. A., and Beuchat, L. R. 1995. J. Food Prot. *58,* 105-107.
12. Dewaal, C. S., Alderton, L., and Jacobson, M. F. 2000. Center for Food Safety and Applied Nutrition. http://vm.cfsan.fda.gov/~dms/.
13. FDA. 2001. Center for Food Safety and Applied Nutrition. http://www.cfsan.fda.gov/~dms/
14. Golden, D. A., Rhodehamel, E. J., and Kautter, D. A. 1993. J. Food Prot. *56*, 194-196.
15. Honnay, R. 1988. Patent 0 255 814.
16. Messer, P. M., Peeler, J. W., and Gilchrist, J. E. 1984. In: FDA Bacteriological Analytical Manual. 6th ed. P 4.01-4.10.
17. NACMCF. 1999. Food Control *10*, 117-143.
18. Nguyen–The, C. and Carlin, F. 1994. Crit. Rev. Food Sci. Nutr. *34*, 371-401.
19. Norris, J.R., and Ribbons, D.W. 1971. In C. Booth (ed.), Methods in Microbiology, Academic Press, London.
20. Ray, B. 1992. In B. Ray and M. Daechel (eds.), *Food Biopreservatives of Microbial Origin*, pp. 209-264, CRC Press, Inc., Boca Raton, FL.
21. Reuter, G. 1985. Int. J. Food Microbiol. *2*, 55-68
22. Richardson, S.D., Thurston, Jr., A.D., Caughran, T.V., Collette, T.W., Patterson, K.S., and Lykins Jr., B.W. 1998. Food Technol. *52*, 58-61.
23. Sapers, G. M., and Simmons G. 1998. Food Technol. *52*, 48-52.
24. Sapers, G. M., Miller, R. L., Pilizota, V., and Mattrazo, A. M. 2001. J. Food Sci. *66*, 345-349.
25. Shewfelt, R.L. 1987. J. Food Qual. *10*, 143-156.
26. Stevens, K. A., Klapes, N.A., Sheldon, B. W., and Klaenhammer, T.R. 1992a. J. Food Prot. *55*, 763-766.
27. Stevens, K. A., Klapes, N.A., Sheldon, B. W., and Klaenhammer, T.R. 1992b. Appl. Environ. Microbiol. *58*, 1786-1788.
28. Stevens, K. A., Klapes, N.A., Sheldon, B. W., and Klaenhammer, T.R. 1991. Appl. Environ. Microbiol. *57,* 3613-3615.
29. Tamplin, M. 1997. Dairy, Food and Environ. Sanitation *17*, 284-286.
30. Ukuku, D. O., Pilizota, A. V., and Sapers, G. M. 2001. J. Food Safety *21*, 31-47.
31. Ukuku, D. O., and Fett, W. F. 2002. J. Food Safety *22,* 231-253.
32. Ukuku, D. O., and Sapers, G. M. 2001. J. Food Prot. *64,* 1286-1291.
33. Wei, C-I., Cook, D.L., and Kirk, J.R. 1985. Food Technol. *1*, 107-115.
34. Wells, J.M., Liao, C.-H., and Hotchkiss, A.T. 1998. Plant Dis. *82*, 491-495.